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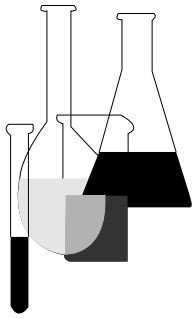
Ecological Effects Test Guidelines

OPPTS 850.4100 Terrestrial Plant Toxicity, Tier I (Seedling Emergence)



“Public Draft”

INTRODUCTION

This guideline is one of a series of test guidelines that have been developed by the Office of Prevention, Pesticides and Toxic Substances, United States Environmental Protection Agency for use in the testing of pesticides and toxic substances, and the development of test data that must be submitted to the Agency for review under Federal regulations.

The Office of Prevention, Pesticides and Toxic Substances (OPPTS) has developed this guideline through a process of harmonization that blended the testing guidance and requirements that existed in the Office of Pollution Prevention and Toxics (OPPT) and appeared in Title 40, Chapter I, Subchapter R of the Code of Federal Regulations (CFR), the Office of Pesticide Programs (OPP) which appeared in publications of the National Technical Information Service (NTIS) and the guidelines published by the Organization for Economic Cooperation and Development (OECD).

The purpose of harmonizing these guidelines into a single set of OPPTS guidelines is to minimize variations among the testing procedures that must be performed to meet the data requirements of the U. S. Environmental Protection Agency under the Toxic Substances Control Act (15 U.S.C. 2601) and the Federal Insecticide, Fungicide and Rodenticide Act (7 U.S.C. 136, *et seq.*).

Public Draft Access Information: This draft guideline is part of a series of related harmonized guidelines that need to be considered as a unit. *For copies:* These guidelines are available electronically from the EPA Public Access Gopher (gopher.epa.gov) under the heading ‘‘Environmental Test Methods and Guidelines’’ or in paper by contacting the OPP Public Docket at (703) 305–5805 or by e-mail: guidelines@epamail.epa.gov.

To Submit Comments: Interested persons are invited to submit comments. By mail: Public Docket and Freedom of Information Section, Office of Pesticide Programs, Field Operations Division (7506C), Environmental Protection Agency, 401 M St. SW., Washington, DC 20460. In person: bring to: Rm. 1132, Crystal Mall #2, 1921 Jefferson Davis Highway, Arlington, VA. Comments may also be submitted electronically by sending electronic mail (e-mail) to: guidelines@epamail.epa.gov.

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OPPTS 850.4100 Terrestrial plant toxicity, Tier I (seedling emergence).

(a) **Scope**—(1) **Applicability.** This guideline is intended to meet testing requirements of both the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136, *et seq.*) and the Toxic Substances Control Act (TSCA) (15 U.S.C. 2601).

(2) **Background.** The source material used in developing this harmonized OPPTS test guideline is OPP 122–1 Seed Germination/Seedling Emergence and Vegetative Vigor (Tier I) (Pesticide Assessment Guidelines, Subdivision J—Hazard Evaluation; Nontarget Plants) EPA report 540/09-82-020, 1982.

(3) **Test objective.** This guideline should be used in conjunction with OPPTS guideline 850.4000, Background—Nontarget plant testing, which provides general information and overall guidance for the nontarget plants test guidelines.

(i) **General.** Seedling emergence studies are designed to provide phytotoxicity data on a pesticide. These phytotoxicity data are needed to evaluate the effect of the level of pesticide exposure to nontarget and terrestrial plants and to assess the impact of pesticides on endangered and threatened plants as noted under the Endangered Species Act. The preliminary level (Tier I) study evaluates the effect of the maximum exposure level. Where a phytotoxic effect is noted in one or more plants, further studies may be required. These studies are required by 40 CFR 158.150 to support the registration of any pesticide intended for outdoor use under FIFRA, as amended.

(ii) **Objective of seedling emergence test, Tier I.** (A) The objective of the Tier I seedling emergence test is to determine if a pesticide exerts a detrimental effect to plants during early critical stages in their development. The test is performed on species from a cross-section of the nontarget terrestrial plant population that have been historically used for this type of testing and, therefore, have known types of responses. This is a maximum dose test designed to evaluate the phytotoxic effects of the pesticide quickly at the one dose.

(B) The terrestrial nontarget plant phytotoxicity seedling emergence test is a greenhouse or growth chamber test. The test organisms are three required species—corn, soybeans, and a root crop, plus seven other species, usually tomato, cucumber, lettuce, cabbage, oat, ryegrass, and onion (six species of at least four families of dicots and four species of at least two families of monocots). The soil or plant surface is treated with test chemical (typical end use product (TEP)) at a concentration comparable to the maximum label application rate or at a concentration 3× the estimated environmental concentration. Results are reported in grams or pounds of active ingredient (AI) per acre and are expressed as the percent

of detrimental effect growth compared to the control after at least 14 days. Parameters measured include plant height, plant dry weight, and percent phytotoxicity. The results are used to establish acute toxicity levels to indicate if further testing at a higher tier is necessary.

(b) **Test standards.** In addition to the general test standards set forth in OPPTS 850.4000, the following standards for the seedling emergence studies apply:

(1) **Test substance.** Refer to 40 CFR part 158 for information required on the test substance.

(2) **Species.** Each report should include the following information:

(i) Identification of the six dicotyledoneae species and four monocotyledoneae species with family identification.

(A) The six dicots are to be of at least four different families and the monocots of at least two families. Soybeans, corn and a dicot root crop like carrot are the required species. The proposed species and families are given below and are acceptable for the seedling emergence test:

Table 1.—Species and families acceptable for the seedling emergence test

Family	Species	Common name
Solanaceae	*Lycopersicon esculentum*	Tomato
Cucurbitaceae	*Cucumis sativus*	Cucumber
Compositae	*Lactuca sativa*	Lettuce
Leguminosae [1]	*Glycine max*	Soybean
Cruciferae	*Brassica oleracea*	Cabbage
Umbelliferae	*Daucus carota*	Carrot
Gramineae	*Avena sativa*	Oat
Gramineae	*Lolium perenne*	Perennial ryegrass
Gramineae	*Zea mays*	Corn
Amaryllidaceae	*Allium cepa*	Onion

[1] Innoculation with *Rhizobium japonicum* is unnecessary

(B) Seeds of plants with a low or variable germination potential should be avoided for the seedling emergence study. When selecting plant species other than corn, soybean, and a root crop, the Agency encourages the use of sensitive plants other than crop plants—weeds, native species, perennial species, etc. The Agency also encourages testing of more than 10 plant species.

(ii) Identification of the cultivars of the plant species or assignment of an identification number to the cultivar used and seed or plant source.

(iii) Identification of the number of replicates and the number of plants per replicate per dose.

(iv) Identification of the date of planting, date of pesticide application and height of plants at application, and date of phytotoxicity rating or harvest and analysis.

(3) **Application levels.** One concentration level equal to no less than maximum label rate should be tested. If it can be determined that the maximum quantity that will be present in the nontarget area is significantly less than the maximum label rate, a concentration equal to no less than 3× that maximum quantity may be tested. The phrase ‘‘the maximum label rate’’ means the maximum recommended amount of AI in the recommended minimum quantity of carrier such as water to be used per land area.

(4) **Number of plants.** At least three replicates, each with 10 plants, should be tested per dose level for the seedling emergence tests. Larger populations and more replicates may be needed to increase the statistical significance of the test.

(5) **Site.** The seedling emergence studies should be conducted under controlled conditions in growth chambers, greenhouses, or in small field plots.

(6) **Duration.** Seedling emergence should be observed weekly, or more frequently, for at least 2 weeks after germination.

(c) **Seedling emergence protocol** The following protocol has been developed to provide guidance in the performance of pesticide plant hazard evaluation testing: Seeds may be germinated in pots using a standardized soil. Three replicates should be set up using at least 10 seeds per pot. The seeds may be surface-sterilized. The soil or support medium should be sprayed or otherwise treated with a known quantity of the chemical, typically the maximum label dosage. The test conditions should approximate those optimal conditions for the species and varieties considered. The seeds should be incubated for at least 14 days. The seeds are observed after 10 and 14 days, and seedling emergence is recorded as the number of emerged seedlings. Shoot height, shoot dry weight, and visual phytotoxicity are also recorded. Root dry weights are also reported for root inhibiting pesticides. See paragraph (g)(1) of this guideline for further guidance.

(d) **Reporting.** In addition to the information required in OPPTS 850.4000, the test report should include the following information.

(1) The number of seeds tested and the number emerged per dosage level for each replicate.

(2) Descriptions of the appearance and the growth and development of the seeds and emergent plants, indicating any abnormalities and expressions of phytotoxicity.

(3) Tabulation of the results indicating the percentage effect level for each species as compared to untreated control plants.

(4) Data on dry weights and heights, or other growth parameters are required to be submitted.

(e) **Tier progression.** If the results of the seedling emergence test have indicated an adverse effect greater than 25 percent on one or more plant species, the seedling emergence tests at the Tier II level are required (see OPPTS 850.4200). If less than a 25 percent detrimental effect or response is noted for the seedling emergence test, no additional testing of the respective tests at higher tiers is ordinarily required. The Agency, after review of the data, may require certain additional tests to determine a more definite nondiscernible effect level.

(f) **Data reporting.** (1) The registrant's report on preliminary seedling emergence studies should include all information necessary to provide:

(i) A complete and accurate description of the laboratory/greenhouse/ field treatments and procedures.

(ii) Sampling data and phytotoxicity rating.

(iii) Data on storage of the plant material if so performed.

(iv) Any chemical analysis of the plant material as to chemical content,

(v) Reporting of the data, rating system, and statistical analysis.

(vi) Quality control measures/precautions taken to ensure the fidelity of the operations.

(2) Each laboratory/greenhouse/small field plot seedling emergence report should include the following information:

(i) **General.** (A) Cooperator or researcher (name and address),test location (county and state; country, if outside of the United States), and date of study.

(B) Name (and signature), title, organization, address, and telephone number of the persons responsible for planning/supervising/ monitoring.

(C) Trial identification number.

(D) Quality assurance indicating control measures/precautions followed to ensure the fidelity of the phytotoxicity determinations; record-keeping procedures, and availability of logbooks; skill of the laboratory personnel; equipment status of the laboratory or greenhouse; degree of adherence to good laboratory practices; and degree of adherence to good agricultural practices in maintaining healthly plants.

(E) Other information the registrant considers appropriate and relevant to provide a complete and thorough description of the test procedures and results.

(ii) **Test substance (pesticide).** (A) Identification of the test pesticide AI including chemical name, common name (ANSI, BSI, ISO, WSSA), and company developmental/experimental name.

(B) AI percentage, plus any inerts and adjuvants in test material.

(C) Solvent used to dissolve and apply the pesticide if the pesticide is insoluble in water or other intended carrier.

(D) Dose rates in terms of AI per area of land or of leaf (if leaf-area-index is provided).

(E) Dose rates in terms of the maximum label rate, or if the registrant has shown that the maximum quantity that will be present in the nontarget area is the maximum label rate, the dose equal to or no less than three times that maximum environmental quantity (environmental quality calculations).

(F) Method of application including equipment type.

(G) Number of applications.

(iii) **Site of the test.** (A) Site description of the seedling emergence and vegetative vigor studies such as the type of growth chamber, greenhouse, or field plot.

(B) Location of the test site.

(C) Climatological data during the test (records of applicable conditions for the type of site, i.e., temperature, thermoperiod, rainfall or water regime, light regimen—intensity and quality, relative humidity, wind speed).

(D) Plant density and container types.

(E) Cultural practices such as cultivation, pest control, and irrigation practices (frequency of watering and method used—overhead vs. bottom watering).

(F) Substrate characteristics (name/designation of soil type and its physical and chemical properties, including pH and percent organic matter).

(iv) **Results.** (A) Reporting of percent emergence, plant height, plant dry weights, root dry weights, root length, dead plants, or other growth parameters that may have been measured to ascertain toxic effects of the

pesticide upon the plants with dates of observation (Root measurements are only needed if the test chemical is a root inhibitor).

(B) Phytotoxicity rating (including a description of the rating system) for each plant or population in the test.

(C) Statistical analysis of the results including an environmental or effective concentration effect (EC) value. (Note, for Tier I, there will be only a percent effect level at a specific concentration which is then compared to 25 percent of the growth (mass or rate] of the control.)

(v) **Evaluation.** (i) For Tier I studies, determination as to whether Tier II studies would be required due to phytotoxic effects noted in one or more of the tested species.

(g) **References.** The following references should be consulted for additional background material on this test guideline.

(1) Truelove, B., ed., *Research Methods in Weed Science.* Southern Weed Science Society. Auburn Printing, Auburn, AL. (1977).

(2) [Reserved]